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ORIGINAL ARTICLE

Behavior of *Phrynocephalus frontalis* to Avoid Traps

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Abstract Previous studies have shown that reptiles are capable of spatial learning and learn to locate important environmental resources so that they can return to those locations at a future time, when needed. Lizards improve their adaptability and survival by learning the position of their shelter in a complex environment. This behavior raises the question, whether lizards can sense danger, such as a trap, in their surroundings, by determining the location of the trap and avoiding it. In the present study, we used a pitfall trap to test if steppe toad-headed agama, *Phrynocephalus frontalis*, can learn to recognize the position of the trap and avoid it. Our results revealed that the percentage of activity time in the trap area was significantly reduced ($P < 0.001$) and the time of drop trap was also significantly reduced ($P = 0.00631$). The number of burrows dug by lizards distributed in the trap area was the least. Reduced activity time in the trap area was observed to have no obvious relationship with the drop in the number of burrows. The present study, therefore, demonstrates that *P. frontalis* are capable of learning the avoidance of a trap by locating its position. The findings offer significant insight in the understanding of reptilian behavior, which is important in the study of the role of reptiles in global ecology, especially because they are often very sensitive to environmental changes.

Keywords reptiles, escape, spatial learning, *Phrynocephalus frontalis*, fitness

1. Introduction

In the study of animal behavior, learning is defined as a process, by which animals assimilate information to correct their behavior. Because of learning, animals adapt to changing environments, better. For individuals, learning is the ability to easily acquire resources and to get away from risks under insecure environments. Juvenile individuals can learn from the experience of their parents to increase their survival rate. In a population, the survival experience gained by individuals spreads fast, helping each group member improve its learning efficiency, and the beneficial experiences are retained by the population to enhance the fitness of individuals (Shang, 2005). In a short time, the significance of learning manifests on the individuals.

Many aspects are involved in the learning behavior of animals. Through learning, they gain the ability and experience to fulfill their requirements and acquire resources around them, such as food, water, and safe shelter, which are necessary for their survival. One important behavioral tool that animals use to accomplish these tasks is spatial learning, an animal's ability to learn the location of vital resources so that it can return to those locations, in future, at a time of need (Paulissen, 2008). Animals, while fulfilling their requirements, are also vulnerable to various risks, such as predators, which might even cost them their lives. Therefore, cognition and timely avoidance of the risks is very important for animals. Learning the location of shelter or escape way to evade predators effectively is significant for survival in all animals.

Few researches about the learning behavior of reptiles have been reported. Earlier studies suggested that reptiles have limited capacity of learning, because they are ectotherms; their activities are restricted by the environmental temperature, and the conclusions drawn could be misleading if we ignore this trait. By the late 20th century, researchers gradually realized that only an appropriate temperature should be given, for reptiles to perform normal physiological activities under experimental conditions (Hertz *et al*., 1982).

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Subsequently, this viewpoint was applied to the studies on the learning behavior of reptiles, showing that spatial learning required more natural stimuli (such as food, light, or heat) at the temperatures appropriate for the species being studied. Several studies have demonstrated that lizards (Day *et al.*, 1999, 2001, 2003; Schall, 2000), snakes (Holtzman *et al.*, 1999), and turtles (Lopez *et al.*, 2001) are capable of spatial learning. Recently, using Keynes maze to test spatial learning, La Dage *et al.* (2012) demonstrated that side-blotched lizard (*Uta stansburiana*) displayed spatial memory and had considerable ability of learning to adjust to a new environment. Moreover, other researchers showed that little brown skinks (*Scincella lateralis*) memorize some significant landmarks in the familiar environment, and effectively locate their shelter to increase their escape rate (Paulissen, 2008).

All the above-mentioned reports suggested that lizards might have the ability of cognizance in their home range and can memorize some specific environmental factors and location by learning. We can extrapolate this premise to suggest that lizards have bias towards beneficial environmental factors, strengthening the assumption that deviation in the behavior can occur by learning and memory. Based on this hypothesis, we attempted to determine whether lizards identify the environment factors (such as a trap) and whether they cognize this kind of disadvantageous condition and strive to avoid it by learning.

Steppe toad-headed agama (*Phrynocephalus frontalis*) lives in arid climate, in places where vegetation is sparse and the ground is exposed and broad. It is widely distributed in the Midwest of Inner Mongolia and its neighboring areas, with high density (Zhao *et al.*, 1999). On being frightened, *P. frontalis* quickly flees, running circuitously, but accurately, into a cave or other safe shelter (Xu *et al.*, 2001). In the present study, we used *P. frontalis* to explore whether it could cognize the position of the trap by learning and memorizing, and acquires the ability to avoid it.

2. Materials and Methods

2.1 Animal collection *P. frontalis* samples were collected from the east of Hobq desert, located in the northern part of Erdos (40°12′N, 111°06′E, 1038.3 m above sea level), Inner Mongolia. Twenty adult male lizards (snout-vent length = 49.92 ± 1.02 mm) were captured by hand. This species is suitable as experimental subject because individuals of this species perform high level of activity, which is beneficial for quick familiarization with the experimental environment. The animals are fast running, good at jumping, and easy to fall into a trap. They are also good at digging caves.

2.2 Fence and pitfall The experiments were conducted in the wild. The sample area belonged to the typical Steppe habitats, where the main vegetation is *Artemisia sphaerocephala* Krasch, which is grown in bare sand and is very favorable for *P. frontalis*. We used drift fences and pitfall traps (Gu and Liang, 2009); eight nearly circular iron fences were made in the open area (perimeter, diameter, and height were 10, 3, and 0.3 m, respectively). Each fence embedded within it a randomly placed trap (a plastic bucket, 20 cm in diameter, 30 cm in depth); the trap was located towards a side, close to the fence. Considering the point of contact of the trap and the fence as the center of the circle, the fence was divided into three parts. The sector with radius 1 m was named part A, that with radius 2 m, excluding part A, was named part B, and the remaining area of the circle was named part C; the parts were marked with small sticks (Figure 1). The whole fence was covered with net to prevent predation. Vegetation inside the fence was partly eliminated for its uniform distribution, maintaining, however, enough food for the lizards, without the need to provide extra food.

2.3 Basic design We observed *P. frontalis* from 08:30 to 12:00 am and from 15:30 to 18:00 pm, the times when it is maximally active. *P. frontalis* were randomly grouped (each group containing four lizards) into batches and only one individual was placed in each fence at a time. The lizards were left alone in the fence for 24 hours before starting the experiment, to familiarize them with the environment in the fence; the trap was covered with lid during this time. After 24 hours, we started the observations and opened the trap, for the duration of the test.

The experiment was setup for about seven days. Each individual was observed on three days (the first, third, and fifth). We proceeded with two groups that were tested at the same time. The steps of the experiment were staggered such that each group was observed on a different day (on the first day we put the individuals of group 1 inside the four fences and allowed them to get familiar with the environment; we started observing group 1 on the second day and put group 2 members into another set of the four fences for familiarizing; these were observed on the third day whereas group 1 was allowed to be free). We observed each individual for 2 hour a day (1 hour between 08:30 and 12:00 am, another between 15:30 and 18:00 pm), and two lizards were observed at the same

time. Before the observation, we checked each fence, and if *P. frontalis* in any of them was dropped into the trap, it was taken out and the record was made. If any lizard dropped into the trap during the test, it was not taken out until the test was over (only a layer of sand was present in the trap as a negative stimulus; no positive inducer was present).

The time during which the lizards stayed in part A, part B, and part C, inside the fence were independently recorded with digital display watches, along with the number of times they dropped into the trap each day during the test. The number of holes dug by the lizards and their locations during the test was also recorded.

2.4 Statistical Analyses We employed Generalized Estimating Equations (GEE; SPSS16.0) to evaluate the time percentage that *P. frontalis* spent in the three parts of the fence during the test. Thereafter, we used Friedman ANOVA to compare the number of times the lizards dropped and the number of burrows distributed in different parts; the significance level was considered as $\alpha = 0.05$.

3. Results

3.1 Analyses of the dropped times We tested 20 individuals during the experiment and observed that the *P. frontalis* dropped 62 times in total and each day the trend of dropped times increased after an initial decrease (Figure 2). Friedman ANOVA revealed that the results were statistically significant ($N = 20$, $\chi^2 = 17.97125$, $df = 6$, $P = 0.00631$).

3.2 Analyses of time percentage The stay time was calculated as time percentage (district activity time/total time) for each individual. The time percentage during the test was plotted to obtain the statistical results (Figure 3). The time percentage that lizards stayed in part A was significantly reduced during the experiment and was statistically significant ($\chi^2 = 44.189$, $df = 2$, $P < 0.001$). Moreover, comparison of the time percentage on the first, second, and third day showed a negative correlation ($B = -0.172$, $B = -0.244$), with an obvious decreasing tendency ($P < 0.001$, $P < 0.001$). A negative correlation was also observed when lizards dropped into the trap ($B = -0.032$), but it was not statistically significant ($P = 0.333$; Table 1).

The time percentage variation was not obvious in part B ($\chi^2 = 2.761$, $df = 2$, $P = 0.251$) Comparison of the time percentage on the first, second, and third day revealed a positive correlation ($B = 0.133$, $B = 0.138$), however, it

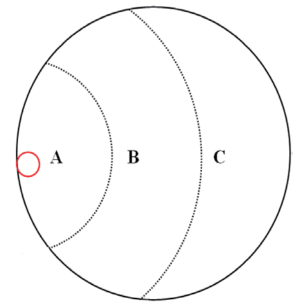

Figure 1 Schematic diagram of the fence (red circle is the trap).

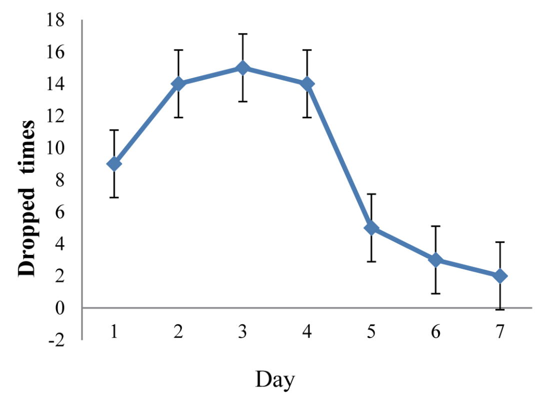

Figure 2 Dropped times of Phrynocephalus frontalis in seven days during the experiment data are expressed as means ± SE. Sample size was 20.

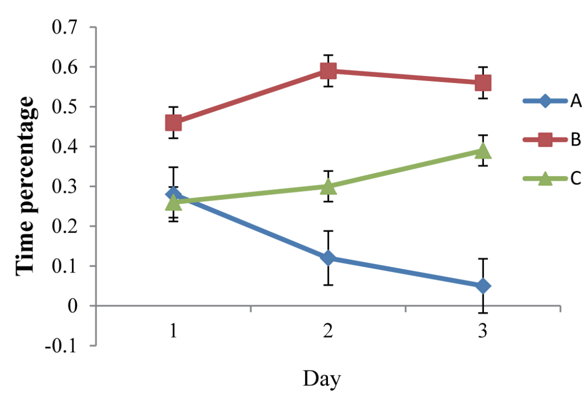

Figure 3 Time percentage of lizards stay in the three parts data are expressed as means ± SE. Sample size was 20.

was not statistically significant ($P = 0.143$, $P = 0.152$). A positive correlation was observed when the lizards dropped ($B = 0.085$), but this too was not statistically significant ($P = 0.320$; Table 2).

The time percentage variation was not obvious in part C, as well, and was also not statistically significant ($\chi^2 = 1.035$, $df = 2$, $P = 0.596$). Comparison of the time percentage on the first, second, and third day revealed a positive correlation ($B = 0.002$, $B = 0.066$), which was not statistically significant ($P = 0.981$, $P = 0.488$). However,

the correlation was negative when the lizards dropped into the trap (B = −0.081), but it was not statistically significant (P = 0.317; Table 3).

3.3 Number and location of the burrow During the experiment, we tested 20 *P. frontalis* individuals, which together dug 45 burrows; the average number of burrows in each part is shown in Figure 4. As shown in this figure, least number of burrows was distributed in part A whereas the number was the maximum for part B. We also used Friedman ANOVA to compare the number of the burrows distributed in different parts. The results obtained were determined to be statistically significant (N = 20, χ^2 = 9.433333, df = 6, P = 0.00895).

4. Discussion

Escaping from the situations of disadvantage is a natural instinct of all animals. On feeling threatened, the most direct and effective recourse for animals is to escape. W.E. Cooper has done a lot of research on the escape behavior of lizards, based on the theory of optimal escape. He has also reported a series of research findings about the factors that affect the escape of lizards from the predation risk; these factors included the distance between predators and prey (Cooper, 1997a, 1997b), the speed of the predator (Cooper, 1997a; Cooper, 1997b), lizard's body temperature, etc. (Cooper, 2003). Research about cognition and escape disadvantages (such as trap) for lizard has not been reported as yet. The present study used the method of drift fences and pitfall traps to investigate the manner in which the toad-headed lizards deal with the trap around their surroundings. The results indicated that *P. frontalis* could recognize and memorize the location of the trap and were capable of learning the avoidance of

Table 1 Parameter estimates, confidence intervals, and hypothesis tests of results in generalized estimating equations (GEE) on part A.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	*df*	Sig.
(Intercept)	0.307	0.0460	0.216	0.397	44.417	1	0.000
Day 3	–0.244	0.0549	–0.352	–0.136	19.774	1	**0.000**
Day 2	–0.172	0.0285	–0.227	–0.116	36.338	1	**0.000**
Day 1	0^a	-	-	-	-	-	-
WD = 1	–0.032	0.0336	–0.098	0.033	0.936	1	0.333
WD = 0	0^a	-	-	-	-	-	-

WD = whether dropped; 1 = dropped, 0 = no.

Table 2 Parameter estimates, confidence intervals, and hypothesis tests of results in generalized estimating equations (GEE) on part B.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	*df*	Sig.
(Intercept)	0.400	0.0954	0.213	0.587	17.569	1	0.000
Day 3	0.137	0.0923	–0.044	0.318	2.213	1	0.137
Day 2	0.144	0.0895	–0.031	0.319	2.598	1	0.107
Day 1	0^a	-	-	-	-	-	-
WD = 1	0.075	0.0811	–0.084	0.234	0.848	1	0.357
WD = 0	0^a	-	-	-	-	-	-

WD = whether dropped; 1 = dropped, 0 = no.

Table 3 Parameter estimates, confidence intervals, and hypothesis tests of results in generalized estimating equations (GEE) on part C.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	*df*	Sig.
(Intercept)	0.334	0.0968	0.144	0.523	11.882	1	0.001
Day 3	0.078	0.0925	–0.104	0.259	0.703	1	0.402
Day 2	0.016	0.0766	–0.134	0.166	0.043	1	0.835
Day 1	0^a	-	-	-	-	-	-
WD = 1	–0.088	0.0788	–0.243	0.066	1.255	1	0.263
WD = 0	0^a	-	-	-	-	-	-

WD = whether dropped; 1 = dropped, 0 = no.

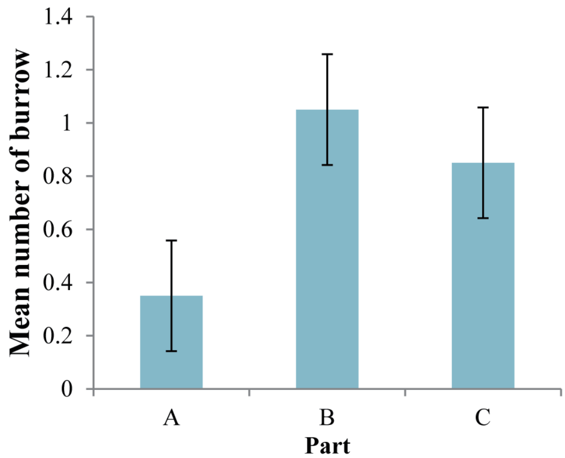

Figure 4 Mean number of burrow distributed in the three parts data are expressed as means ± SE. Sample size was 45.

the trap by rapidly determining its position; however, this behavior was independent of the fact whether the lizards dropped or not.

The difference in time percentage of lizard activity in part B and part C was not obvious from the experimental results. The reason for this observation could be related to some individuals with decreased activity during the test. It could be that the personality types of such lizards were negative or "slow." The term "personality" describes behavioral differences among individuals that are consistent over time or contexts, for example, in the tendency to approach novel objects or predators, explore new environments, or interact with conspecifics (Gosling, 2001). In a few studies comparing fitness of personality types over multiple years, there is evidence that "slow" personality types, that is, less exploratory, neophobic, less active, or passive animals are less successful than "fast" (exploratory, neophilic, active, or aggressive) types in relatively stable environments but that this is reversed in changeable environments (Dingemanse and Réale, 2005). Those individuals differ from the usual and stay in the hole or nearby for more than half of the observation time when we were observing them, which constantly happened in the middle and later periods of the experiment.

Intriguingly, the time, lizards stayed in part C, showed negative correlation with whether they were off-trap (B = 0.0788). The design of experimental drift fences was nearly circular, which might have confused the small lizards into losing their orientation. *P. frontalis* continually jumped and climbed along with the fence, trying to escape. However, because of the pitfall tangent with the fence, the lizards had the possibility of falling-off, no matter which direction they chose to climb. This format of fence and trap arrangement could have conditioned a reflex response in the lizards after they dropped several times and could have led to the cognition of proximity to the fence as a danger. Some reptiles show similar phenomena; for example, juvenile corn snakes use a white card taped to the side of the test arena to help them orient to a retreat (Holtzman *et al.*, 1999). Although part C was farthest from the trap area, it contained most of the fence. Perhaps, lizards could significantly reduce their activity in part A after the stimulus of drop. It might also be possible that due to the conditioned reflex to the fence and the fall into the trap, the lizards kept away from the fence and chose to stay in part B, where most part was in the middle of the fence.

In summary, our results suggest that *P. frontalis* have strong ability to cognize the trap by locating its position, adapt to the complex environment, and have higher fitness. Since research on spatial learning and memory is limited in reptiles, the findings offer significant insight in the understanding of reptilian behavior, important in the study of the role of reptiles in global ecology.

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